EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on Flavouring Group Evaluation 93, Revision 1 (FGE.93Rev1)

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2013.3452

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2013;11(11):3452

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 93, Revision 1 (FGE.93Rev1)

Consideration of sulphur containing heterocyclic compounds evaluated by JECFA (68th meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives evaluated by EFSA in FGE.21Rev3[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of five sulphur-containing heterocyclic compounds [FL-no: 15.010, 15.126, 15.128, 15.130 and 15.131] evaluated by the JECFA at its 68th meeting in 2007. This revision is required owing to additional available genotoxicity data on 2-acetyl-2-thiazoline [FL-no: 15.010]. Since the publication of FGE.93, the substance [FL-no: FL-no: 15.127] is no longer supported by Industry for use as a flavouring substance in Europe and will therefore not be considered any further. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The two substances 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131], which are 3-thiazolines, are structural similar to two other 3-thiazolines in FGE.21Rev1 for which the Panel has expressed a genotoxicity concern, and accordingly the Procedure should not be applied to these two substances until adequate genotoxicity data become available. The Panel agrees with the application of the Procedure as performed by the JECFA for the remaining three substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 3-(methylthio)-methylthiophene [FL-no: 15.126] and 2-propionyl-2-thiazoline [FL-no: 15.128], of the five substances considered in this FGE and agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. Besides the safety assessment of these

[1] On request from the European Commission, Question No EFSA-Q-2013-00180, EFSA-Q-2013-00182, EFSA-Q-2013-00183, EFSA-Q-2013-00184 adopted on 24 October 2013.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Maria de Fatima Tavares Poças, Fidel Toldra and Detlef Wölfle. Correspondence: cef@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby and EFSA staff: Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on Flavouring Group Evaluation 93, Revision 1 (FGE.93Rev1). EFSA Journal 2013;11(11):3452, 42 pp. doi:10.2903/j.efsa.2013.3452

Available online: www.efsa.europa.eu/efsajournal

flavouring substances, the specifications for the materials of commerce have also been considered and for all five substances, the information is adequate.

KEY WORDS

thiazolines, sulphur containing substances, FGE.21, FGE.93, JECFA 68th meeting, food safety

SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the CEF Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

In Flavouring Group Evaluation 93 (FGE.93), the EFSA considered six sulphur containing heterocyclic substances evaluated by the JECFA (68th meeting). This revision is made due to additional available genotoxicity data on 2-acetyl-2-thiazoline [FL-no: 15.010], as requested in previous version of FGE.93. For one substance [FL-no: 15.127], additional toxicity data ware requested in FGE.93. However, since publication of FGE.93 the substance [FL-no: 15.127] is no longer supported by Industry for use as a flavouring substance in Europe and will therefore not be considered any further. Therefore, the present revision of FGE.93, FGE.93Rev1, considers five flavouring substances evaluated by the JECFA.

The Panel concluded that the five substances in the JECFA flavouring group of sulphur-containing heterocyclic substances are structurally related to the 59 substances evaluated by EFSA in FGE.21Rev3.

The Panel agrees with the way the application of the Procedure was applied by the JECFA for three of the five substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 3-(methylthio)-methylthiophene [FL-no: 15.126] and 2-propionyl-2-thiazoline [FL-no: 15.128]. The remaining two substances, 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131], which are 3-thiazolines, are structurally similar to two other 3-thiazolines in FGE.21 for which the Panel has expressed a genotoxicity concern, and accordingly the Procedure should not be applied to these two substances until adequate genotoxicity data become available.

Genotoxicity data have become available for 2-acetyl-2-thiazoline [FL-no: 15.010] and based on these new *in vitro* studies (gene mutation test in bacteria and micronucleus assay in human peripheral blood lymphocytes), the genotoxicity concern could be ruled out. 2-Acetyl-2-thiazoline [FL-no: 15.010] is supporting the other 2-thiazoline included in this FGE, 2-propionyl-2-thiazoline [FL-no: 15.128], thus the two substances can now be evaluated using the Procedure.

For 2-acetyl-2-thiazoline [FL-no: 15.010] and 2-propionyl-2-thiazoline [FL-no: 15.128] (2-thiazolines), the intakes (MSDI) of 0.51 and 0.19 µg/*capita*/day are below the threshold for their structural class II. The no observed adverse effect level (NOAEL) of 1.8 mg/kg body weight (bw) per day for 2-acetyl-2-thiazoline from a 90-day and a 52-week rat studies that examined a mixture of four flavouring substances, including [FL-no: 15.010], provides margins of safety of 2.1 x 10^5 and 5.7 x 10^5, respectively, in relation to the estimated levels of exposure from their use as flavouring substances. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be concluded at step B4 in the Procedure to be of no safety concern.

For 3-(methylthio)methylthiophene [FL-no: 15.126] the maximised survay-derived daily intake (MSDI) is below the threshold for its structural class (Cramer class III, 90 µg/person/day). The Panel agrees with the JECFA that the NOAEL of 0.29 mg/kg bw/day for the supporting substance 2-thienyl disulphide [FL-no: 15.008] is adequate for [FL-no: 15.126] and that it provides a sufficient safety margin. It can therefore be concluded that this substance is of no safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

For the three substances [FL-no: 15.010, 15.126 and 15.128] evaluated through the Procedure, use levels have been provided by the Industry. The modified theoretical added maximumdaily intake (mTAMDI) figures calculated for the substances [FL-no: 15.010 and 15.128] in structural class II are 810 and 62 µg/person/day, respectively. For substance [FL-no: 15.010] the value exceeds the threshold of 540 µg/person/day for structural class II. For the substance [FL-no: 15.126] in structural class III the mTAMDI figure is 3.8 µg/person/day, which is below the threshold of concern of 90 µg/person/day. Although the two remaining substances [FL-no: 15.130 and 15.131] cannot be evaluated through the Procedure, the corresponding available use levels were considered. For the two substances in structural class III the figures are 160 µg/person/day, which exceeds the threshold of concern of 90 µg /person/day for the structural class. Thus, for three substances [FL-no: 15.010, 15.130 and 15.131] the intakes, estimated on the basis of the mTAMDI approach, exceed the threshold for their structural classes. Therefore more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be considered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusion for the five JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all five JECFA-evaluated substances.

Thus, the Panel concluded that the three substances [FL-no: 15.010, 15.126 and 15.128] are of no safety concern at the estimated intake. For the remaining two substances [FL-no: 15.130 and 15.131] the Panel concluded that the Procedure could not be applied pending submission and evaluation of genotoxicity data.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA has evaluated the flavouring substance 2-methyl-2-thiazoline [FL-no: 15.086] in the Flavouring Group Evaluation 21 (FGE.21). The opinion was adopted on 8 February 2007. EFSA concluded in its opinion that adequate genotoxicity data is required.

EFSA has considered the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluation of six sulphur containing hetereocyclic substances in the Flavouring Group Evaluation 93 (FGE.93). The Opinion was adopted on 23 July 2009. EFSA concluded in its opinion that for four substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] adequate genotoxicity data is required.

The requested information on one representative material, 2-acetyl-2-thiazoline [FL-no: 15.010] has now been submitted by the European Flavour Association. This information is intended to cover re-evaluation of this substance and of the three substances [FL-no: 15.128, 15.130 and 15.131] in FGE.93Rev1. In addition, it should cover the re-evaluation of 2-methyl-2-thiazoline [FL-no: 15.086] from FGE.21.

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substances.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests EFSA to carry out a safety assessment on the following five flavouring substances: 2-acetyl-2-thiazoline [FL-no: 15.010], 2-methyl-2-thiazoline [FL-no: 15.086], 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] in accordance with Commission Regulation (EC) N° 1565/2000.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. Official Journal of the European Communities 31.12.2008, L 354/34-50.

[5] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. Official Journal of the European Communities 2.10.2012, L 267, 1-161.OJ L 267, 2.10.2012, p. 1.

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000, hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the maximised survey-derived daily intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided to also perform an estimate of the daily intakes per person using a modified theoretical added maximum Ddaily Intake (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram (μg)/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional

information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 µg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 µg per day?")" (JECFA, 1999).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 µg per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

1. HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

In FGE.93, which contains a group of six flavouring substances consisting of sulphur-containing heterocyclic substances, the Panel concluded that for four substances [FL-no: 15.010, 15.128, 15.130 and 15.131], the Procedure should not be applied until adequate genotoxicity data become available and for one substance [FL-no: 15.127] lack of toxicity information prevented its final evaluation through the Procedure.

Industry has informed that the substance 1-(3-hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127] is no longer supported for use as a flavouring substance in Europe (EFSA, 2011) and the substance will therefore not be considered any further.

FGE	Opinion adopted	Link	No. of substances
FGE.93	23 July 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1206.htm	6
FGE.93Rev1	24 October 2013		5

The present revision of FGE.93 (FGE.93Rev1) concerns the re-consideration of the four JECFA-evaluated substances [FL-no: 15.010, 15.128, 15.130 and 15.131] considered in FGE.93.

Additional data, genotoxicity studies (*in vitro* reverse mutation and micronucleus assays) on 2-acetyl-2-thiazoline [FL-no: 15.010] and new tonnage figures for two substances [FL-no: 15.010 and 15.128] (EFFA, 2012) have now become available. Furthermore, new information from Industry on missing stereoisomeric composition for [FL-no: 15.130 and 15.131] (EFFA, 2013a) and information on specifications for [FL-no: 15.126 and 15.128] (EFFA, 2013b) is also included in the present revision.

In the document on "Representative substances for testing" (EFSA CEF Panel, 2012a), substance 2-acetyl-2-thiazoline [FL-no: 15.010] was identified as a supporting substance with respect to genotoxicity for 2-methyl-2-thiazoline [FL-no: 15.086] included in FGE.21, and for 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] included in FGE.93. However, [FL-no: 15.130 and FL-no: 15.131] which in the document "Representative substances for testing" were grouped with 2-thiazolines are in fact 3-thiazolines and should have been grouped together with 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029], 4,5-dimethyl-2-ethyl-3-thiazoline [FL-no: 15.030] and 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] included in FGE.76 and together with 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119] included in FGE.21. For the group of 3-thiazolines, 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] was identified as a supporting substance with respect to genotoxicity. Therefore, 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] included in FGE.93, will be evaluated by the Panel together with the other 3-thiazolines when the requested genotoxicity studies on 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] become available.

2. PRESENTATION OF THE SUBSTANCES IN THE JECFA FLAVOURING GROUP

2.1. Description

2.1.1. JECFA Status

The JECFA has evaluated a group of 17 flavouring substances consisting of sulphur-containing heterocyclic substances at its 68th meeting (JECFA, 2007; JECFA, 2008a).

2.1.2. EFSA Considerations

The current FGE.93Rev1 deals with five [FL-no: 15.010, 15.126, 15.128, 15.130, 15.131] of the 17 substances evaluated by the JECFA (JECFA, 2008a).

- Seven of the substances evaluated by the JECFA in 2008 are not in the Register [2-(4-methyl-5-thiazolyl)ethyl formate, 2-(4-methyl-5-thiazolyl)ethyl propionate, 2-(4-methyl-5-thiazolyl)ethyl butanoate, 2-(4-methyl-5-thiazolyl)ethyl isobutyrate, 2-(4-methyl-5-thiazolyl)ethyl hexanoate, 2-(4-methyl-5-thiazolyl)ethyl octanoate, 2-(4-methyl-5-thiazolyl)ethyl decanoate (JECFA-no: 1751-1757).
- Four other substances have been evaluated by the AFC Panel of EFSA (before the JECFA) in FGE.21 [FL-no: 15.063, 15.055, 15.076 and 15.114] (JECFA-no: 1758, 1763, 1764 and 1766).
- Industry has informed that substance [FL-no: 15.127] (JECFA-no: 1750) is no longer supported for use as a flavouring substance in Europe and the substance will therefore not be considered any further.

This consideration therefore only deals with five substances [FL-no: 15.010, 15.126, 15.128, 15.130, 15.131].

The Panel concluded that these five substances of the JECFA flavouring group of sulphur containing heterocyclic substances are structurally related to the group of thiazoles, thiophene, thiazoline and thienyl derivatives evaluated by EFSA in the Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3)[7] (EFSA CEF Panel, 2012). The substances in FGE.21Rev3 were subdivided into a number of subgroups. The five substances in the current FGE.93Rev1 are assigned to the following two FGE.21 subgroups:

[7] The Panel is aware that for FGE.21, a revision 4 has been released. For the candidate substances in subgroups B-II and B-III of FGE.21Rev3, a concern with respect to genotoxicity was raised. This concern is also applicable to candidate substances [FL no: 15.130 and 15.131] in FGE.93. Since in revision 4 of FGE.21 subgroup B-III has been removed, in order to facilitate the identification of the reason for this concern for these two substances in FGE.93, reference to FGE.21Rev3 is maintained, rather than to FGE.21Rev4.

- 3-(Methylthio)-methylthiophen [FL-no: 15.126] in subgroup A-Ic.
- 2-Acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] in subgroup B-II.

2.2. Isomers

2.2.1. Status

The following two substances [FL-no: 15.130 and 15.131] in the group of the JECFA evaluated sulphur containing heterocyclic substances have chiral centres.

2.2.2. EFSA Considerations

Adequate information on isomeric composition is available for the two isomeric substances [FL-no: 15.130 and 15.131] (Table 1).

2.3. Specifications

2.3.1. Status

The European Flavour Industry has submitted specifications for the substances commercially used in Europe (EFFA, 2006a; EFFA, 2006b; Flavour Industry, 2004; Flavour Industry, 2005). Although the JECFA specifications are available, the specifications used in this consideration are those submitted by the Industry (See Table 3).

2.3.2. EFSA Considerations

Specifications including complete purity criteria and identity tests are available for all substances.

3. INTAKE ESTIMATION

3.1. JECFA Status

For all five substances evaluated through the JECFA Procedure intake data (MSDI) are available for EU.

3.2. EFSA Considerations

For all the JECFA-evaluated substances normal and maximum use levels have been provided by the Flavour Industry [FL-no: 15.010, 15.126, 15.128, 15.130 and 15.131] (EFFA, 2006a; EFFA, 2006b; EFFA, 2007; Flavour Industry, 2005) (see Table 1). Based on these normal use levels, mTAMDI figures (see Table 2) can be calculated. For definition of normal and maximum use levels and description of the method for calculation of mTAMDI consult Annex II in e.g. (EFSA, 2004).

Table 1: Normal and Maximum use levels (mg/kg) available for the JECFA evaluated substances in FGE.93Rev1

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
15.010	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	4 2	0,2 1	4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	4 2	1 5	0,2 1
15.126	0,01 0,1	0,01 0,1	- -	0,005 0,05	- -	- -	- -	0,02 0,2	0,005 0,05	- -	- -	- -	0,005 0,05	- -	- -	- -	0,05 0,5	0,01 0,1
15.128	0,16 0,8	0,04 0,2	0,16 0,8	- -	- -	0,04 0,2	0,01 0,05	0,4 1	0,04 0,4	- -	- -	- -	0,08 0,8	- -	0,01 0,08	0,04 0,2	0,08 0,8	- -
15.130	-	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2

	-	1,1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.131	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1

Table 2: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
15.010	2-Acetyl-2-thiazoline	0.51	ND	810	Class II	540
15.127	1-(3-Hydroxy-5-methyl-2-thienyl)ethanone	0.012	ND	ND	Class II	540
15.128	2-Propionyl-2-thiazoline	0.19	ND	62	Class II	540
15.126	3-(Methylthio)-methylthiophen	0.012	ND	3.8	Class III	90
15.130	5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline	0.012	ND	160	Class III	90
15.131	5-Ethyl-4-methyl-2-(2-butyl)-thiazoline	0.012	ND	160	Class III	90

ND) Not determined.

SUMMARY OF SPECIFICATION DATA

Table 3: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2008b)

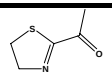
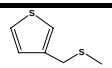
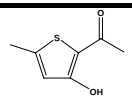
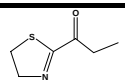
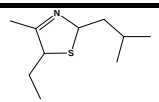
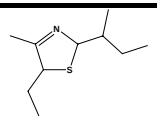
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
15.010 1759	2-Acetyl-2-thiazoline		3817 2335 29926-41-8	Solid C_5H_7NOS 129.18	Practically insoluble or insoluble Soluble	 27 IR NMR MS 98 %	n.a. n.a.	(EFFA, 2006b).
15.126 1765	3-(Methylthio)-methylthiophen		 61675-72-7	Liquid $C_6H_8S_2$ 144.26	Slightly soluble Slightly soluble	210-211 n.a. IR NMR MS 97 %	1.580-1.586 1.522-1.528	(EFFA, 2006a). Register name to be changed to 3-(methylthio)-methylthiophene.
15.127 1750	1-(3-Hydroxy-5-methyl-2-thienyl)ethanone		4142 133860-42-1	Solid $C_7H_8O_2S$ 156.2	Slightly soluble Soluble	 74.3 (969 hPa) IR NMR MS 98.3 %	n.a. n.a.	(Flavour Industry, 2004). Substance no longer supported by Industry (EFSA, 2011).
15.128 1760	2-Propionyl-2-thiazoline		4064 29926-42-9	Liquid C_6H_9NOS 143.21	Insoluble Soluble	237-241 IR NMR MS 99 %	1.510-1.525 1.130-1.330	(EFFA, 2006a).
15.130 1761	5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline		4319 83418-53-5	Liquid $C_{10}H_{19}NS$ 185.33	Soluble Soluble	253 NMR MS 95 %	1.483-1.489 0.939-0.945	(Flavour Industry, 2005). Industry: cis- and trans-5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline. Mixture of diastereoisomers, each of them racemic (EFFA, 2013a).
15.131 1762	5-Ethyl-4-methyl-2-(2-butyl)-thiazoline		4318 83418-54-6	Liquid $C_{10}H_{19}NS$ 185.33	Soluble Soluble	253 NMR MS 95 %	1.487-1.493 0.950-0.956	(Flavour Industry, 2005). Mixture of diastereoisomers, each

Table 3: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2008b)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
								of them racemic (EFFA, 2013a).

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

4. GENOTOXICITY DATA

4.1. Genotoxicity Studies – Text Taken[8] from the JECFA Report (JECFA, 2008a)

Thiophene [FL-no: 15.106] (structurally related substance)

The results of several *in vitro* tests for genotoxicity conducted with thiophene, a structurally related substance, are described below.

No increase in mutagenic activity was observed in the reverse mutation assay (Ames test) in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537 at 0, 78.1, 156, 313, 625, 1250, 2500 or 5000 µg thiophene/plate with and without metabolic activation. Toxicity was noted at 1500 µg/plate in TA1537 and at 2500 µg/plate in TA98, TA100 and TA1535 (Shibuya, 2006).

Similarly, there was no increase in mutagenic activity in a mutation assay in *Escherichia coli* strain WP2uvrA at 0, 313, 625, 1250, 2500 or 5000 µg/plate with and without metabolic activation. Toxicity was noted at the 5000 µg/plate concentration (Shibuya, 2006).

There was no increase in chromosomal aberrations or polyploidy following incubation of Chinese hamster lung cells with 0, 210, 420 or 840 µg thiophene/ml (Tanaka, 2006).

Conclusion on genotoxicity

No genotoxicity data were available on the 17 sulphur containing hetereocyclic substances evaluated by the JECFA at its 68th meeting. The JECFA evaluation refers to data on thiophene only, as summarised in Table 4. The JECFA concluded that in its previous evaluation of substances in this group (JECFA, 2003), studies on genotoxicity, as well as studies on acute toxicity and short-term toxicity were available and none raised safety concerns.

4.2. Genotoxicity Studies – Text Taken[9] from EFSA FGE.21Rev3 (EFSA, 2012b)

Genotoxicity data were provided for 12 candidate substances. These 12 substances belong to subgroup A-Ia: thiophene [FL-no: 15.106]; subgroup A-Ib: 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092], 2,5-dimethylthiophene [FL-no: 15.064], 2-acetylthiophene [FL-no: 15.040], 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]; subgroup A-II: 2,4-dimethylthiazole [FL-no: 15.062]; subgroup A-III: 2-methyl-4,5-benzothiazole [FL-no: 15.088]; subgroup B-III: 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099]. There were also mutagenicity data on four supporting substances and on four other structurally related substances. All available information on genotoxicity of the 12 candidate and the four supporting substances and of four other structurally related substances is based upon *in vitro* studies only.

In the following text from FGE.21Rev3 (EFSA, 2012b), only the information for subgroup A-I, B-I, B-II and B-III has been presented, as the information for subgroups A-II and A-III, B-IV, B-V and B-VI was not relevant to the candidate substances in the current FGE.

Subgroup A-I: Thiophenes
Thiophene [FL-no: 15.106], 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092] and 2,5-dimethylthiophene [FL-no: 15.064] were reported to be negative in microbial mutagenicity assays. 2-Acetylthiophene [FL-no: 15.040] was negative in microbial tests, using *Salmonella typhimurium strains* TA98 and TA100, with and without metabolic activation and in the SOS chromotest with metabolic activation. 2-Acetylthiophene was reported to be positive without metabolic activation in the SOS *Escherichia coli* chromotest (Mosier et al., 2003). In the same study,

[8] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*
[9] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107] and 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] gave positive results without metabolic activation in the SOS *E. coli* chromotest. The concentrations tested were not reported for any of the substances subjected to the SOS *E. coli* chromotest (Mosier et al., 2003). The Panel considered the endpoint of this test inappropriate for the estimation of genotoxic potential. The supporting substance 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] was negative in a microbial mutagenicity assay.

Thiophene was tested in accordance to OECD guidelines in a bacterial reverse mutation test in strains of *S. typhimurium* and in strain WP2 uvrA of *E. coli*. No evidence of mutagenic response was reported when strains TA100, TA1535, TA98 and TA1537 of *S. typhimurium* were incubated at concentrations of 0, 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/plate with and without S9 metabolic activation. Toxicity was observed at 1250 µg/plate in TA1537, and 2500 µg/plate in strains TA100, TA1535 and TA98 also with and without metabolic activation. Toxicity was observed at 5000 µg/plate in WP2 with and without S9 metabolic activation (Shibuya, 2006).

In a chromosomal aberration test, thiophene was tested on Chinese hamster lung cells in accordance with Japanese Guidelines. No chromosomal aberrations or polyploidy was reported when incubated with concentrations of 0, 210, 420, 840 µg/mL of thiophene, with and without metabolic activity (Tanaka, 2006).

Subgroups B-I and B-II: Dihydrothiophenes and thiazolines

No genotoxicity information was available for any candidate or supporting substances in these subgroups. However, considering the structural similarities between the thiazolines in subgroup B-II and the thiazolidines in subgroup B-III, the Panel also concluded that the thiazolines [FL-no: 15.060, 15.086 and 15.119] could not be evaluated through the Procedure (see Subgroup B-III below).

Subgroup B-III: Thiazolidines

The two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as well as the structurally related ethyl, isopropyl, n-butyl and isobutyl thiazolidine have all been reported to be positive in the Ames tests (TA98 and TA100) (Mihara and Shibamoto, 1980). Owing to limited reporting, the data could not be properly evaluated. Nevertheless, these reports do raise the possibility of a genotoxic potential of these thiazolidines. Accordingly, it was concluded not to evaluate the candidate substances 2-methylthiazolidine and 2-propylthiazolidine through the Procedure.

Conclusion on genotoxicity

It is concluded that the genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in the present revision of FGE.21, Revision 3. However, except for the two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], the two thiazolidines 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and the three structurally related thiazolines 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 49 candidate substances using the Procedure.

For a summary of *in vitro* genotoxicity data considered by EFSA, see Table 4.

4.3. New Genotoxicity Studies on 2-Acetyl-2-thiazoline [FL-no: 15.010]

In vitro

2-Acetyl-2-thiazoline [FL-no: 15.010] was tested for the induction of gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 both in the presence and absence of

Aroclor 1254 induced rat liver S9-mix. Two independent experiments were carried out (Mc Garry, 2012).

In the first experiment, 2-acetyl-2-thiazoline was tested in all five strains in the absence and presence of S9-mix using the plate incorporation methodology at concentrations of 5, 15.8, 50, 158.1, 500, 1581 and 5000 µg/plate. No evidence of toxicity was observed in any strains at any concentration tested.

In the second experiment, the concentrations of 2-acetyl-2-thiazoline used in all strains in the absence and presence of S9-mix were 156.3, 312.5, 625.0, 1250, 2500 and 5000 µg/plate of 2-acetyl-2-thiazoline, and the pre-incubation method was used for treatments in the presence of S-9. No evidence of toxicity was observed in any strains at any concentration tested.

No statistically significant increases in revertant numbers were observed in any of the tester strains that were both concentration-related and clearly reproducible.

It is concluded that 2-acetyl-2-thiazoline [FL-no: 15.010] did not induce mutations in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under the conditions of the study (Table 5). The study was conducted using GLP and the design complied with current recommendations (OECD Guideline 471) and an acceptable top concentration was achieved.

In vitro micronucleus assay

2-Acetyl-2-thiazoline [FL-no: 15.010] was tested for the induction of chromosome damage and potential aneugenic effects in an *in vitro* micronucleus assay using duplicate human peripheral blood lymphocytes prepared from pooled blood from two healthy male volunteers. Treatments were performed both in the absence and presence of Aroclor 1254 induced rat liver S9-mix (Watters, 2012).

Cells were stimulated for 48 hours with phytohaemaglutinin to produce exponentially growing cells.

A preliminary toxicity range-finding experiment was conducted with S9-mix and 3 hours treatment and without S9-mix with 3 and 24 hours treatment. Toxicity was evaluated as the effect of treatment on the Replication Index (RI). Twelve concentrations from 4.6 to 1262 µg/mL were tested. The concentrations selected for the main experiment were based on toxicity data from this preliminary test.

In the main experiment, cells were treated for 3 hours, followed by 21 hours recovery (3 + 21 hours), with 0, 600, 1000 or 1292 µg/ml of 2-acetyl-2-thiazoline in the absence and in the presence of S9-mix. The levels of toxicity (reduction in RI) at the top concentration were 15 % and 0 % in the absence and presence of S-9, respectively. In a parallel assay, cells were treated for 24 hours (24 + 0 hours) with 0, 100, 200, 400 or 600 µg/ml of 2-acetyl-2-thiazoline in the absence of S9-mix with no recovery period. The top concentration induced 55 % toxicity. Relevant positive and negative controls were included in all experiments. There were 2 replicate cultures per treatment, and 1000 binucleate cells per replicate (i.e., 2000 cells per dose) were scored for micronuclei. Thus, the study was conducted under GLP and the design complies with current recommendations (including OECD Guideline 487). No evidence of chromosomal damage or aneuploidy was observed as indicated by the lack of increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of rat liver S9 metabolic activation (Table 5).

In conclusion, 2-acetyl-2-thiazoline [FL-no: 15.010] did not induce micronuclei in male human peripheral blood lymphocyte cultures when tested for 3 + 21 hours in the presence of S9-mix and at up to toxic concentrations for 3 + 21 hours and 24 + 0 hours in the absence of S9-mix.

For a summary of *in vitro* genotoxicity data on 2-acetyl-2-thiazoline [FL-no: 15.010], see Table 5.

4.4. EFSA Considerations

No genotoxicity information was available on the 17 substances when evaluated by the JECFA in 2008 (JECFA, 2008a), including the five substances considered in this Opinion. However, new *in vitro* genotoxicity studies (gene mutation test in bacteria and micronucleus assay in human peripheral blood lymphocytes) have become available on 2-acetyl-2-thiazoline [FL-no: 15.010], which is considered to be supporting for the other 2-thiazoline included in this FGE, 2-propionyl-2-thiazoline [FL-no: 15.128]. 2-Acetyl-2-thiazoline [FL-no: 15.010] was negative in both assays and the Panel therefore concluded that 2-acetyl-2-thiazoline [FL-no: 15.010] and 2-propionyl-2-thiazoline [FL-no: 15.128] do not give rise to concern with respect to genotoxicity and can accordingly be evaluated using the Procedure.

Two of these FGE.93 substances, 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131], which are 3-thiazolines, are structurally similar to two other 3-thiazolines in FGE.21Rev1 for which the Panel has expressed a genotoxicity concern, and accordingly, the Procedure should not be applied to these two substances until adequate genotoxicity data become available.

For the remaining substance 3-(methylthio)-methylthiophene [FL-no: 15.126], supported by FGE.21 subgroup A-Ic, since no genotoxicity data were available on the substance, the Panel referred to the EFSA conclusion on the substances considered in group A-Ic in FGE.21Rev3 (EFSA, 2012b). For this subgroup A-Ic, EFSA had concluded that genotoxicity data were limited, but the data did not preclude evaluating the substances through the Procedure. The Panel concluded therefore, that the available data (from FGE.21Rev3) do not preclude taking 3-(methylthio)-methylthiophene [FL-no: 15.126] through the Procedure (done in previous version of FGE.93).

5. APPLICATION OF THE PROCEDURE

5.1. Application of the Procedure to Sulphur Containing Heterocyclic Compounds by the JECFA (JECFA, 2008a)

According to JECFA, two of the substances belong to structural class II, and three to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

All five substances were concluded at step B4 in the JECFA Procedure – i.e. that the substances are not expected to be metabolised to innocuous products and that the estimated intakes are below the thresholds for their structural classes II and III. An adequate NOAEL was available for relevant structurally related substances for all five substances and the JECFA concluded that the substances are therefore not expected to be of safety concern when used as flavouring substances.

In conclusion, the JECFA evaluated all five substances to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the five sulphur containing heterocyclic substances are summarised in Table 6: Summary of Safety Evaluation of Sulphur Containing Heterocyclic Compounds (JECFA, 2008a))

5.2. Application of the Procedure to 59 Thiazoles, Thiophene, Thiazoline and Thienyl Derivatives and Miscellaneous Substances from Chemical Group 30 by EFSA (FGE.21Rev3) (EFSA, 2012b)

Fifty-nine candidate substances were evaluated in FGE.21Rev3. Forty-eight substances were classified into structural class II and 11 into structural class III using the decision tree approach presented by Cramer et al. (1978).

For seven substances the Procedure could not be applied due to indication of genotoxic potential *in vitro* [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133].

The substances were allocated into structural subgroups (for description and explanation, see FGE.21Rev3 (EFSA, 2012b) and were evaluated at step B4 in the Procedure, i.e. the substances are not expected to be metabolised to innocuous products and the estimated intakes are below the thresholds for their structural classes II and III.

In summary, the Panel concluded that 26 of the candidate substances evaluated through the Procedure, from the structural subgroups A-Ic (thiophenes with thiol-containing ring substituents) and A-II (thiazoles) are not of safety concern at their estimated levels of intake based on the MSDI approach, whereas for 26 candidate substances from the structural subgroups A-Ia (thiophene), A-Ib (thiophenes with non-thiol-containing ring substituents), A-III (benzothiazoles), B-I (dihydrothiophenes), B-IV (dithiazines) and B-VI (thiadiazine) additional data are required.

The stepwise evaluations of the 59 substances are summarised in Table 7: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.21Rev3) (EFSA, 2012b).

5.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure was applied by the JECFA for three of the five sulphur containing heterocyclic substances [FL-no: 15.010, 15.126 and 15.128].

Two of these five substances, 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131], which are 3-thiazolines, are structurally similar to two other 3-thiazolines in FGE.21Rev1 for which the Panel has expressed a genotoxicity concern, and accordingly the Procedure should not be applied to these two substances until adequate genotoxicity data become available.

For 2-acetyl-2-thiazoline [FL-no: 15.010] and 2-propionyl-2-thiazoline [FL-no: 15.128], which are 2-thiazolines, the intakes (MSDI) of 0.51 and 0.19 µg/*capita*/day are below the threshold for their structural class II. The NOAEL of 1.8 mg/kg bw per day for 2-acetyl-2-thiazoline from a 90-day and a 52-week rat studies that examined a mixture of four flavouring substances including [FL-no: 15.010] (Munday and Kirkby, 1971; Munday and Kirkby, 1973) provides margins of safety of 2.1 x 10^5 and 5.7 x 10^5 respectively, in relation to the estimated levels of exposure from their use as flavouring substances. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be concluded at step B4 in the Procedure to be of no safety concern. The studied mixture included also 3-hydroxy-4,5-dimethylfuran-2(5H)-one [Fl.no: 10.030] and was used by the Panel to support the substances in FGE.10Rev3 (EFSA CEF Panel, 2012c).

For 3-(methylthio)-methylthiophene [FL-no: 15.126], allocated to subgroup A-Ic, the intake (MSDI) of 0.12 µg/*capita*/day is below the threshold for its structural class III. The Panel agrees with the JECFA, and in line with the conclusion reached by EFSA for subgroup A-Ic in FGE.21Rev3, that an adequate NOAEL provides a sufficient safety margin and that this flavouring substance can be concluded at step B4 in the Procedure as of no safety concern.

CONCLUSION

In Flavouring Group Evaluation 93 (FGE.93), the EFSA considered six flavouring substances from a group of flavouring substances consisting of sulphur-containing heterocyclic substances evaluated by the JECFA at its 68th meeting. This revision is made due to additional available genotoxicity data on 2-acetyl-2-thiazoline [FL-no: 15.010], as requested in previous version of FGE.93. For one substance [FL-no: 15.127], additional toxicity data was requested in FGE.93. However, since publication of FGE.93, the substance [FL-no: 15.127] is no longer supported by Industry for use as a flavouring substance in Europe and will therefore not be considered any further. Therefore, the present revision of FGE.93, FGE.93Rev1, considers five flavouring substances evaluated by the JECFA.

The Panel concluded that all the five substances from the JECFA flavouring group of sulphur containing heterocyclic substances are structurally related to the 59 substances evaluated by EFSA in FGE.21Rev3.

The Panel agrees with the way the application of the Procedure was applied by the JECFA for three of the five substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 3-(methylthio)-methylthiophene [FL-no: 15.126] and 2-propionyl-2-thiazoline [FL-no: 15.128]. The remaining two substances, 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131], which are 3-thiazolines, are structurally similar to two other 3-thiazolines in FGE.21 for which the Panel has expressed a genotoxicity concern, and accordingly the Procedure should not be applied to these two substances until adequate genotoxicity data become available.

Genotoxicity data have become available for 2-acetyl-2-thiazoline [FL-no: 15.010] and based on these new *in vitro* studies (gene mutation test in bacteria and micronucleus assay in human peripheral blood lymphocytes), the genotoxicity concern could be ruled out. 2-Acetyl-2-thiazoline [FL-no: 15.010] is supporting the other 2-thiazoline included in this FGE, 2-propionyl-2-thiazoline [FL-no: 15.128], so the two substances can now be evaluated using the Procedure.

For 2-acetyl-2-thiazoline [FL-no: 15.010] and 2-propionyl-2-thiazoline [FL-no: 15.128] (2-thiazolines) the intakes (MSDI) of 0.51 and 0.19 µg /*capita*/day are below the threshold for their structural class II. The NOAEL of 1.8 mg/kg bw per day for 2-acetyl-2-thiazoline from a 90-day and a 52-week rat studies that examined a mixture of four flavouring substances, including [FL-no: 15.010], provides margins of safety of 2.1 x 10^5 and 5.7 x 10^5, respectively, in relation to the estimated levels of exposure from their use as flavouring substances. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

For 3-(methylthio)methylthiophene [FL-no: 15.126], the Maximised Survey-derived Daily Intake (MSDI) is below the threshold for its structural class (Cramer class III, 90 µg/person/day). The Panel agrees with the JECFA that the NOAEL of 0.29 mg/kg bw/day for the supporting substance 2-thienyl disulfide [FL-no: 15.008] is adequate for [FL-no: 15.126] and that it provides a sufficient safety margin. It can therefore be concluded that this substance is of no safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

For the three substances [FL-no: 15.010, 15.126 and 15.128] evaluated through the Procedure, use levels have been provided by the Industry. The mTAMDI figures calculated for the substances [FL-no: 15.010 and 15.128] in structural class II are 810 and 62 µg/person/day, respectively. For substance [FL-no: 15.010] the value exceeds the threshold of 540 µg/person/day for structural class II. For the substance [FL-no: 15.126] in structural class III the mTAMDI figure is 3.8 µg/person/day, which is below the threshold of concern of 90 µg/person/day. Although the two remaining substances [FL-no: 15.130 and 15.131] cannot be evaluated through the Procedure, the corresponding available use levels were considered. For the two substances in structural class III the figures are 160 µg/person/day, which exceeds the threshold of concern of 90 microgram/person/day for its structural class. Thus, for three substances [FL-no: 15.010, 15.130 and 15.131] the intakes, estimated on the basis of the mTAMDI approach, exceed the threshold for their structural classes. Therefore more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be considered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusion for the five JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for all JECFA-evaluated substances.

Thus, the Panel concluded that three substances [FL-no: 15.010, 15.126 and 15.128] are of no safety concern at the estimated intake. For the remaining two substances [FL-no: 15.130 and 15.131] the

Panel concluded that the Procedure could not be applied pending submission and evaluation of genotoxicity data.

SUMMARY OF GENOTOXICITY DATA

Table 4: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA, 2012b)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
Subgroup A-Ia						
Thiophene [15.106]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (all strains) (252 µg/plate)	Negative (±S9)	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test with three strains, quantitative study (4 doses, 0.03, 0.3, 3, 30 µmol/plate) with TA100 only. Limited report of experimental details and results. Insufficient quality, study not considered adequate for the evaluation of mutagenic activity.
	Ames assay (preincubation method)	*S. typhimurium* TA97;TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate	Negative (±S9)[1]	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD Guideline 471. The study is considered valid.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01-1.2 mmol/plate (100,968 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by ”mutation factor,” no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (8414 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
	Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 78.1, 156, 313, 625, 1250 µg/plate	Negative (±S9)	(Shibuya, 2006)	Valid study according to OECD Test Guidelines and Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
		E. coli WP2 uvrA	0, 78.1, 156, 313, 625, 1250, 2500, 5000 µg/plate	Negative (±S9)		
	Chromosomal Abberation	Chinese hamster lung cells	0, 210, 420, 840 µg/ml	Negative (±S9)	(Tanaka, 2006)	Valid study according to Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
Subgroup A-Ib						
2-Methylthiophene [15.091]	Ames assay (preincubation	*S. typhimurium* TA98; TA100;	0.00001 - 1.0 mmol/plate	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by ”mutation factor,” no numbers are given for negative

Table 4: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA, 2012b)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
	method)	TA102	(98,170 µg/plate)			results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
3-Methylthiophene [15.092]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01-1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by ”mutation factor,” no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
2,5-Dimethylthiophene [15.064]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11,219 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
2-Acetylthiophene [15.040]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
2-Acetyl-3-Methylthiophene [15.037]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
Thiophene-2-carbaldehyde [15.107]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
5-Ethylthiophene-2-carbaldehyde [15.074]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.

Table 4: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA, 2012b)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
Subgroup A-II						
2,4-Dimethylthiazole [15.062]	Ames assay (plate incorporation method)	*S. typhimurium* TA100	9.3 and 94 mmol/l top agar (10,639 µg/ml)	Negative (-S9)	(Voogd et al., 1983)	Insufficient quality (one test strain as well as without metabolic activation only).
(4,5-Dimethylthiazole [15.017])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11,318 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
(4-Methylthiazole [15.035])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9916 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
Subgroup A-III						
2-Methyl-4,5-benzothiazole [15.088]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102; TA1535; TA1537	100-10,000 µg/plate	Negative (±S9)[1]	(Longfellow, 1998)	Summary report of NCI-short-term test program, results not given in detail.
(Benzothiazole [15.016])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative (±S9)	(Bayer, 1991)	Summary in IUCLID data set only. According to this summary, the assay was in compliance with GLP; accordance with OECD Guideline 471 not stated.
	Mouse lymphoma assay	Mouse L5178Y tk+/- cells	10-250 µg/ml	Negative (±S9)	(Longfellow, 1997)	Summary report of NCI-short-term test program, results not given in detail.
Subgroup B-III						
2-Propylthiazolidine [15.099]	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA98 and TA100.(±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
2-Methylthiazolidine [15.090]	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100; (±S9) 100 µg/ml: positive	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

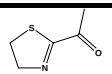
Table 4: Genotoxicity Data (*in vitro*) EFSA / FGE.21Rev3 (EFSA, 2012b)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
				in TA98 and TA100 (±S9)		
(2-Ethylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (±S9) and TA98 (-S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive TA98 and TA100.(±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Isopropylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Butylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (+S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Isobutylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA98 and TA100 (+S9); 10 µg/ml: positive in TA98 and TA100 (±S9); 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

NR: Not reported.

[1] With and without rat and hamster S9 metabolic activation.

Table 5: Summary of Additional Genotoxicity Data on 2-Acetyl-2-thiazoline Submitted by Industry

FL-no JECFA -no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference	Comments
15.010 1759	2-Acetyl-2-thiazoline		Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102	5, 15.81, 50, 158.1, 500, 1581 and 5000 µg/plate[1]	Negative	(Mc Garry, 2012)	Valid study conducted according to current guidelines.
					156.3, 312.5, 625.0, 1250, 2500 and 5000 µg /plate[1,2]	Negative		Valid study conducted according to current guidelines.
			Micronucleus induction	Human peripheral blood lymphocytes	600, 1000 and 1292 µg /ml[3]	Negative	(Watters, 2012)	Valid study conducted according to current guidelines.
					600, 1000 and 1292 µg /ml[4]			
					100, 200, 400 and 600 µg /ml[5]	Negative		Valid study conducted according to current guidelines.

[1] With and without metabolic activation.
[2] Assay modified with pre-incubation in the presence of S9.
[3] Without metabolic activation, 3 hours treatment +21 hours recovery.
[4] With metabolic activation, 3 hours treatment + 21 hours recovery.
[5] Without metabolic activation, 24 hours + 0 hours recovery.

SUMMARY OF SAFETY EVALUATIONS

Table 6: Summary of Safety Evaluation by the JECFA (JECFA, 2008a)

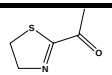
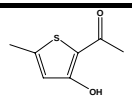
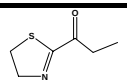
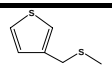
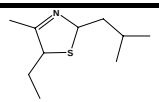
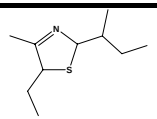
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
15.010 1759	2-Acetyl-2-thiazoline		0.51 ND	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.127 1750	1-(3-Hydroxy-5-methyl-2-thienyl)ethanone		0.012 ND	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No longer supported by Industry, EFSA 2011.	No longer supported by Industry (EFSA 2011).
15.128 1760	2-Propionyl-2-thiazoline		0.19 ND	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
15.126 1765	3-(Methylthio)-methylthiophen		0.012 ND	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. Register name to be changed to 3-(methylthio)-methylthiophene.

Table 6: Summary of Safety Evaluation by the JECFA (JECFA, 2008a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
15.130 1761	5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline		0.012 ND	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Genotoxicity data required.	
15.131 1762	5-Ethyl-4-methyl-2-(2-butyl)-thiazoline		0.012 ND	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Genotoxicity data required.	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
ND not determined.

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

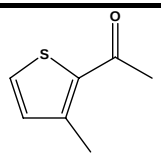
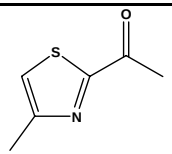
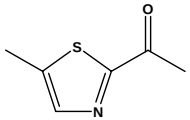
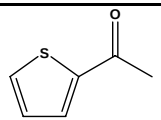
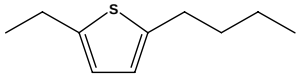
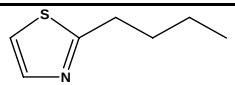
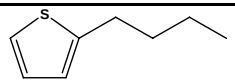
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.037	2-Acetyl-3-methylthiophene		0.18	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.038	2-Acetyl-4-methylthiazole		0.0049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.039	2-Acetyl-5-methylthiazole		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.040	2-Acetylthiophene		2.2	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.043	2-Butyl-5-ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.044	2-Butylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.045	2-Butylthiophene		0.012	Class II B3: Intake below threshold,	Additional data required		

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

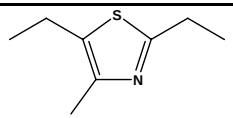
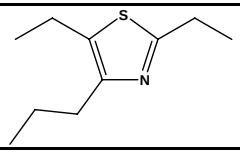
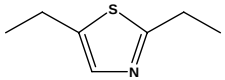
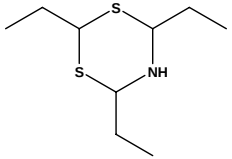
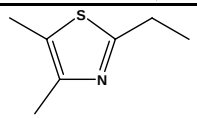
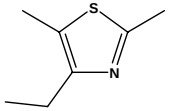
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
				B4: No adequate NOAEL			
15.050	2,5-Diethyl-4-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.051	2,5-Diethyl-4-propylthiazole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.052	2,5-Diethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.058	4,5-Dimethyl-2-ethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.061	2,5-Dimethyl-4-ethylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

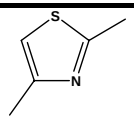
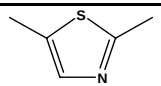
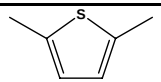
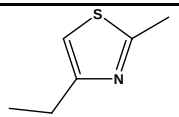
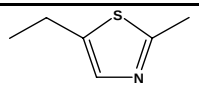
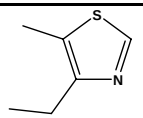
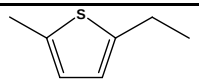
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.062	2,4-Dimethylthiazole		0.61	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.063	2,5-Dimethylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.064	2,5-Dimethylthiophene		0.23	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.067	4-Ethyl-2-methylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.068	5-Ethyl-2-methylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.069	4-Ethyl-5-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.070	2-Ethyl-5-methylthiophene		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

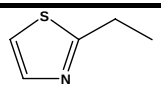
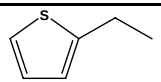
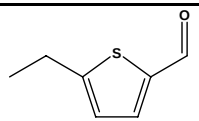
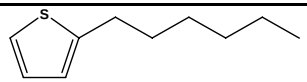
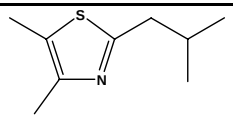
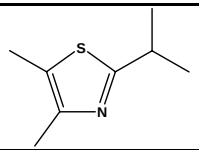
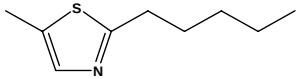
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.071	2-Ethylthiazole		0.028	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.072	2-Ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.074	5-Ethylthiophene-2-carbaldehyde		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.076	2-Hexylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.078	2-Isobutyl-4,5-dimethylthiazole		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.080	2-Isopropyl-4,5-dimethylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.084	5-Methyl-2-pentylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate	4)	6)	

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

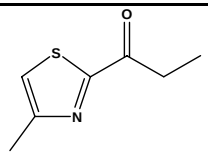
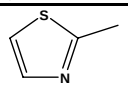
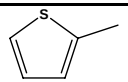
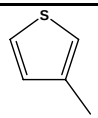
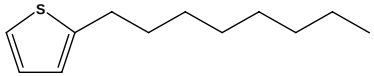
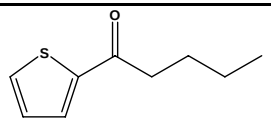
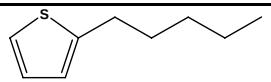
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
				NOAEL exists			
15.085	4-Methyl-2-propionylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.089	2-Methylthiazole		0.018	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.091	2-Methylthiophene		0.019	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.092	3-Methylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.093	2-Octylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.094	2-Pentanoylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.096	sec-Pentylthiophene		0.24	Class II B3: Intake below threshold,	Additional data required		

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

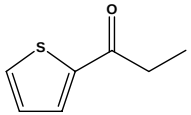
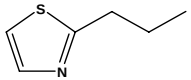
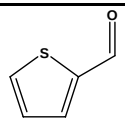
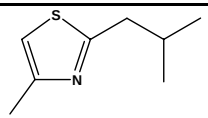
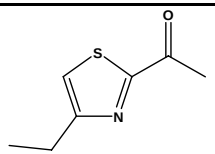
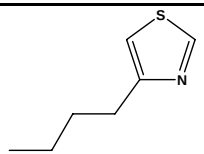
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
				B4: No adequate NOAEL			
15.097	2-Propionylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.098	2-Propylthiazole		0.085	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.107	Thiophene-2-carbaldehyde		0.21	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.115	2-Isobutyl-4-methyl thiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.116	2-Acetyl-4-ethylthiazole		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.118	4-Butylthiazole		1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

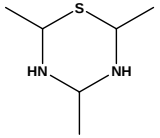
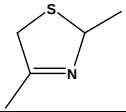
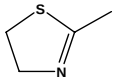
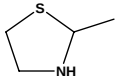
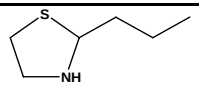
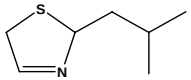
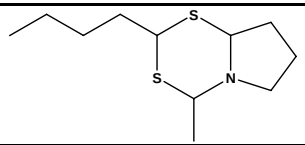
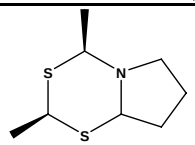
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		0.61	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.060	2,4-Dimethyl-3-thiazoline		0.012	Class II No evaluation			b)
15.086	2-Methyl-2-thiazoline		0.24	Class II No evaluation			b)
15.090	2-Methylthiazolidine		0.024	Class II No evaluation			c)
15.099	2-Propylthiazolidine		0.012	Class II No evaluation			c)
15.119	2-Isobutyl-3-thiazoline		0.011	Class II No evaluation			b)
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine		0.0012	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine		0.055	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

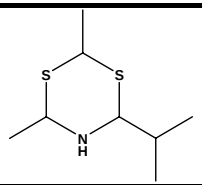
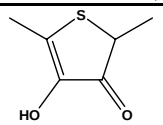
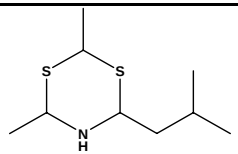
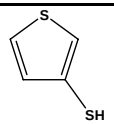
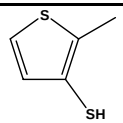
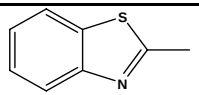
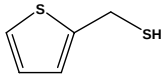
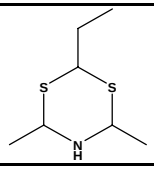
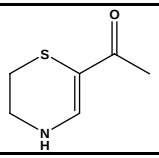
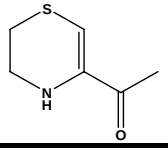
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		1.5	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		5.7	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.082	3-Mercaptothiophene		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.087	2-Methyl-3-mercaptothiophene		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.088	2-Methyl-4,5-benzothiazole		0.0085	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.106	Thiophene		0.12	Class III B3: Intake below threshold,	Additional data required		

Table 7: Summary of Safety Evaluation by the EFSA (FGE.21Rev3) (EFSA CEF Panel, 2012b)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
				B4: No adequate NOAEL			
15.108	2-Thiophenemethanethiol		0.0073	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.135	Ethyl thialdine		0.61	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine		0.012	Class III No evaluation			c)
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine		0.61	Class III No evaluation			c)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/*capita*/day.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

a) Substance not supported by Industry (EFFA, 2009).
b) Genotoxic potential *in vitro*.
c) Genotoxic potential *in vitro*. Substance not supported by Industry (EFFA, 2009).

REFERENCES

Aeschbacher HU, Wolleb U, Loliger J, Spadone JC and Liardon R, 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food and Chemical Toxicology 27(4), 227-232.

Bayer AG, 1991. Report no. 20847, 26 November. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 95-16-9, EINECS Name benzothialzole. Section 1.0.1-5.11.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16(3), 255-276.

EFFA (European Flavour and Fragrance Association), 2006a. Addendum of 2 Flavouring Substance (Candidate Chemicals) of the Flavouring Group Evaluation of the Chemical Group 29 (Annex I of 1565/2000/EC) Structurally Related to Sulfur Containing Heterocyclic and Heteroaromatic Derivatives [FAO/WHO JECFAFAS 50/59] used as Flavouring Substances.Addendum to FGE.21 Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.109.

EFFA (European Flavour and Fragrance Association), 2006b. Transfer files for FGE.93 concerning [FL-no: 15.010]. Unpublished data from EFFA to FLAVIS Secretariat.

EFFA (European Flavour and Fragrance Association), 2007. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.

EFFA (European Flavour Association), 2009. Supplement list of EU-only Footnote-10 materials for Commission. Unpublished communication submitted by EFFA to the FLAVIS secretariat. 14 December 2009.

EFFA (European Flavour Association), 2012. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 29 (Annex I of 1565/2000/EC) Consideration of sulphur containing heterocyclic compounds evaluated by JECFA (68th meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives evaluated by EFSA in FGE.21Rev1(2009). Addendum to FGE.93. May 2012. FLAVIS/8.175.

EFFA (European Flavour Association), 2013a. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 6 March and 25 October 2013. Information on substances evaluated in FGE.21Rev4, FGE.25Rev3, FGE.76Rev1, FGE.90Rev1, FGE.93Rev1. FLAVIS/8.185.

EFFA (European Flavour Association), 2013b. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 19 April 2013. Information on three substances evaluated in FGE.93Rev1 [FL-no: 15.126, 15.127 and 15.128]. FLAVIS/8.187.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012a. Scientific Opinion. Statement on List of Representative Substances for Testing. EFSA Journal 2012;10(3):2639, 9 pp. doi:10.2903/j.efsa.2012.2639

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012b. Scientific Opinion on Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3): Thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30. EFSA Journal 2012;10(2):2457, 95 pp. doi:10.2903/j.efsa.2012.2457

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids) , 2012c. Scientific Opinion on Flavouring Group Evaluation 10, Revision 3 (FGE.10Rev3): Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30. EFSA Journal 2012;10(3):2563, 127 pp. doi:10.2903/j.efsa.2012.2563

EFSA (European Food Safety Authority), 2004. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) on a request from the Commission related to Flavouring Group Evaluation 03 (FGE.03): Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an orthoester of formic acid, from chemical groups 1 and 2. EFSA Journal 2004, 107, 1-59.

EFSA, 2011. List of substances for which the Commission withdraw its request to EFSA for an opinion. FLAVIS/2.23Rev1.

Flavour Industry, 2004. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-93.

Flavour Industry, 2005. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-93.

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Safety evaluation of certain food additives. Fifty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Sixty-seventh Meeting. Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007. Evaluation of certain food additives. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 947. Geneva, 19-28 June 2007.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008a. Safety evaluation of certain food additives and contaminants. Sixty-eight Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 59. IPCS, WHO, Geneva 2008.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008b. JECFA Online Edition "Specification for Flavourings" http://www.fao.org/ag/agn/jecfa-flav/search.html (May, 2008).

Lee H, Bian SS and Chen YL, 1994. Genotoxicity of 1,3-dithiane and 1,4-dithiane in the CHO/SCE assay and the Salmonella/microsomal test. Mutation Research 321, 213-218.

Longfellow D, 1997. Mutagenicity studies. Benzothiazole. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute.

Longfellow D, 1998. Mutagenicity studies. 2-Methylbenzothiazole. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute.

Mc Garry S, 2012. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 2-Acetyl-2-thiazoline. Covance Laboratories Ltd. Study no. 8252828. October 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Mihara S and Shibamoto T, 1980. Mutagenicity of products obtained from cysteamineglucose browning model systems. Journal of Agricultural and Food Chemistry 28(1), 62-66.

Mosier PD, Jurs PC, Custer LL, Durham SK and Pearl GM, 2003. Predicting the genotoxicity of thiophene derivatives from molecular structure. Chemical Research in Toxicology 16(6), 721-732.

Munday R and Kirkby WW, 1971. Biological evaluation of a flavor cocktail. 2. 13-Week study in rats. Research report PCW 71 1624. Unpublished data submitted by EFFA to SCF.

Munday R and Kirkby WW, 1973. Biological evaluation of a flavor cocktail. 3. One-year feeding study in rats. Research report PCW73 1103. Unpublished data submitted by EFFA to SCF.

SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Shibuya T, 2006. Reverse mutation test of thiophene on bacteria. Kanagawa, Japan, Hatano Research Institute, Food and Drug Safety Center.

Tanaka N, 2006. *In vitro* chromosomal aberration test of thiophene on cultured chinese hamster cells. Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan. [

Voogd CE, van der Stel JJ and Verharen HW, 1983. The capacity of some nitro- and amino-heterocyclic sulfur compounds to induce base-pair substitutions. Mutation Research 118, 153-165.

Watters G, 2012. Induction of micronuclei in cultured human peripheral blood lymphocytes. 2-Acetyl-2-thiazoline. Covance Laboratories Ltd. Study no. 8266505. December 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K and Speck W, 1987. Salmonella mutagenicity tests. 3. Results from the testing of 255 chemicals. Environmental and Molecular Mutagenesis 9(Suppl. 9), 1-110.

ABBREVIATIONS

BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practice
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	Micronucleated Binucleate cells
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
RI	Replication Index

SCF	Scientific Committee on Food
WHO	World Health Organisation